Pure Red Cell Aplasia of Long Duration Complicating Major ABO-Incompatible Bone Marrow Transplantation

By Jürg P. Gmürr, Johanna Burger, Andreas Schaffner, Klaus Neftel, Oswald Oelz, Daniel Frey, and Margrit Metaxas

In 3 of 15 consecutive patients receiving a human leukocyte antigen (HLA)-identical but major ABO incompatible bone marrow transplant (BMT), pure red cell aplasia (PRA) lasting 5 to 8 months was observed. Titers of the incompatible anti-A agglutinin before infusion of the red blood cell (RBC)-depleted BMT was very high in one, and in the usual range in two patients. Decrease of agglutinin titers during the first 4 weeks after BMT were comparable between PRA patients and those of ABO-incompatible BMT recipients with timely RBC recovery. However, in PRA patients, agglutinin titers rose again and remained elevated for 19 to 28 weeks. RBC engraftment and reticulocyte recovery ultimately occurred spontaneously and coincided with the decrease of agglutinin titers below 16. These observations indicate that PRA is antibody-dependent in this setting. Furthermore, it is conceivable that cyclosporine facilitates recipient-derived antibody synthesis after major ABO-incompatible BMT.

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M A J O R A B O incompatibility between donor and recipient is not a barrier for successful bone marrow transplantation (BMT). Most studies have demonstrated no increased incidence of graft rejection or early graft failure. Furthermore, no delay in engraftment compared with recipients of ABO-identical marrow was observed.1,8 This is because early red blood cell (RBC) precursors express ABH antigens to a lesser extent, and pluriptotent marrow stem cells presumably not at all.9,10 However, prolonged reticulocytopenia and/or increased dependence on RBC support was reported.3,4,8 Moreover, it is necessary to avoid the risk of acute hemolysis of the large amount of donor RBC trapped with unmodified marrow grafts. This is reliably achieved by reducing the ABO antibody level in the recipient pretransplant2 or by depleting the marrow graft from donor RBC before infusion.3,4,6,8 Despite these precautions significant immunohematologic problems can occasionally occur in the posttransplant period, such as protracted regeneration of all peripheral blood cell counts,11 temporary erythroid hypoplasia,4,12,13 overt hemolysis,3,4,6,8 or graft failure.14

We present three cases (of a total of 15 major ABO-incompatible BMT) with extremely long-lasting pure red cell aplasia (PRA), which ultimately resolved spontaneously several months after BMT. In an attempt to find characteristics predicting such an unusual course, we analyzed several serologic parameters in these patients and patients with normal RBC recovery after ABO-incompatible BMT.

M A T E R I A L S A N D M E T H O D S

During 1977 to 1987, 15 patients underwent major ABO-incompatible BMT: two for severe aplastic anemia (SAA), six for acute myelocytic leukemia (AML), three each for acute lymphocytic (ALL) and chronic myelogenous leukemia (CML), and one for chronic granulomatous disease. Fourteen donors were genotypically human leukocyte antigen (HLA)-identical siblings and one was the one haplotype mismatched father (unique patient number [UPN] 06). Conditioning regimens included cyclophosphamide (Cy; 120 mg/kg) or polychemotherapy (UPN 23 and 50) followed by 10 to 12 Gy total body irradiation in all patients except UPN 02, who received Cy alone (200 mg/kg). For prophylaxis of graft-versus-host disease (GVHD) six patients received methotrexate and nine received cyclosporine, according to standard protocols (Table 1).

Prevention of hemolysis. To avoid the risk of acute hemolytic reaction to marrow infusion, eight patients were subjected to a large-volume plasma exchange followed by 1 U of donor-type RBC on the day of BMT (designated day 0). In seven patients the donor marrow was depleted of RBC by the use of hydroxyethyl starch sedimentation, according to Dinsmore et al.7 After BMT, transfusion support consisted of the administration of packed or washed RBC of recipient-type and random single donor platelets of donor-type.

Laboratory evaluation. All patients had daily leukocyte, platelet, hemoglobin, and hematocrit determinations (Coounter Counter S plus), and at least twice-weekly granulocyte and reticulocyte counts until hospital discharge (days 38 to 56). Subsequently these parameters were assessed weekly up to day 100, and bimonthly afterwards. Bone marrow aspirates were obtained at least once between days 16 and 45, on day 100, and quarterly thereafter. Cyclosporine whole blood trough levels were determined weekly by a commercially available radioimmunoassay.15 ABO and Rhesus typing, alloantibody screening, identification of complete (NaCl 20°C and 37°C) and incomplete anti-RBC alloantibodies (antiglobulin test; 37°C and one-stage bromeline technique), direct antiglobulin testing (broad spectrum antihuman globulin, monospecific anti-immunoglobulin G [IgG], and anti-C3 reagents), and RBC antibody elution (ether technique) were performed by standard methods.16 Commercial reagents (Ortho Diagnostics, Baxter) were used according to the manufacturer's instructions.

Anti-A and anti-B agglutinins were determined by incubating a 5% standard A- and B-RBC suspension in saline with serial dilutions of the recipient's serum for 5 minutes at 20°C followed by centrifugation and scoring for macroscopic agglutination. Anti-A and anti-B IgG antibody titers were determined after dithiothreitol pretreatment of the patient's serum followed by indirect antiglobulin testing at 37°C.17 Agglutinin titers were determined before and after plasma exchange, immediately before BMT and one to two times a week thereafter until discharge, complete disappearance of agglutinins, or definite reticulocyte recovery, whichever took place first. Thereafter, determinations usually were performed weekly until day 100, and one to two times a month until RBC engraftment.

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Table 1. ABO Blood Group of Donors and Recipients, Method Used for Prevention of Hemolysis, GVH Prophylaxis, and RBC Engraftment in 15 Major ABO-Incompatible BMT

<table>
<thead>
<tr>
<th>No. of Days to Achieve</th>
<th>Prevention of Hemolysis By</th>
<th>GVH Prophylaxis</th>
<th>RBC Engraftment</th>
</tr>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>Patients with pure RBC aplasia</td>
<td>RBC depletion</td>
<td>Cyclosporine</td>
<td>247</td>
</tr>
<tr>
<td>39 A O</td>
<td>RBC depletion</td>
<td>Cyclosporine</td>
<td>165</td>
</tr>
<tr>
<td>40 A O</td>
<td>RBC depletion</td>
<td>Cyclosporine</td>
<td>148</td>
</tr>
<tr>
<td>Patients with timely RBC engraftment</td>
<td>Plasma exchange</td>
<td>Methotrexate</td>
<td>NE</td>
</tr>
<tr>
<td>02 B A</td>
<td>Plasma exchange</td>
<td>Methotrexate</td>
<td>22</td>
</tr>
<tr>
<td>09 B A</td>
<td>Plasma exchange</td>
<td>Methotrexate</td>
<td>17</td>
</tr>
<tr>
<td>00 B A</td>
<td>Plasma exchange</td>
<td>Methotrexate</td>
<td>36</td>
</tr>
<tr>
<td>06 B A</td>
<td>Plasma exchange</td>
<td>Methotrexate</td>
<td>26</td>
</tr>
<tr>
<td>10 A O</td>
<td>Plasma exchange</td>
<td>Methotrexate</td>
<td>31</td>
</tr>
<tr>
<td>12 A O</td>
<td>Plasma exchange</td>
<td>Cyclosporine</td>
<td>15</td>
</tr>
<tr>
<td>15 A O</td>
<td>Plasma exchange</td>
<td>Cyclosporine</td>
<td>18</td>
</tr>
<tr>
<td>23 A O</td>
<td>RBC depletion</td>
<td>Cyclosporine</td>
<td>16</td>
</tr>
<tr>
<td>34 A O</td>
<td>RBC depletion</td>
<td>Cyclosporine</td>
<td>35</td>
</tr>
<tr>
<td>66 A O</td>
<td>RBC depletion</td>
<td>Cyclosporine</td>
<td>15</td>
</tr>
<tr>
<td>41 B A</td>
<td>RBC depletion</td>
<td>Cyclosporine</td>
<td>16</td>
</tr>
</tbody>
</table>

Abbreviations: R, reticulocytes; EP, bone marrow erythropoiesis, NE: not evaluable; ND, not done.

For investigation of mixed chimerism after BMT, the sex chromosome was analyzed in bone marrow samples in the sex mismatched case UPN 39. In addition, minisatellite DNA probes were used in UPNs 39 and 40 for identification of donor and recipient origin of hematopoietic cells after BMT. Preliminary mixing experiments showed that 5% to 10% of cells of different parentage could be recognized by this method. For statistical evaluation, Fischer’s exact test and linear regression analysis were applied.

RESULTS

Between 1977 and December 1987, 15 patients received a major ABO-incompatible BMT, corresponding to 23% of all BMT performed at our institution. Details concerning ABO incompatibility, prevention of hemolysis, and GVH prophylaxis are shown in Table 1. Besides transient fever in 7 of 15 patients (±38.5°C, <6 hours), the infusion of the ABO-incompatible marrow was well-tolerated by all patients. No overt hemolysis, change in blood pressure, or renal function impairment was observed.

Bone marrow engraftment. One patient with SAA (UPN 02, Table 1) did not engraft and eventually demonstrated complete recovery of hematopoiesis of recipient-type. In this patient several risk factors for early graft failure were present: multiple transfusions and refractoriness to random platelets before BMT, conditioning with Cy alone, no cyclosporine for GVH prophylaxis, low marrow cell dose of 2 × 10^8/kg, and sex mismatch.

In 14 patients, prompt and durable engraftment could be documented by rapidly rising blood cell counts, sex chromosome markers, and/or ABH blood group antigens. In 11 of 14 patients, timely complete recovery of all three cell lines was observed (Table 2, lower portion), and bone marrow analysis (days 22 through 45 after BMT) showed ≥15% RBC precursors. The remaining three patients (UPNs 39, 40, and 50) showed a comparable speed of engraftment, as determined by rising leukocyte, granulocyte, and platelet counts, but the engraftment was incomplete with significant delay of RBC recovery mimicking the picture of long-lasting PRA (Table 2, upper portion). No comparable delay of RBC engraftment was observed in 52 of 53 consecutive ABO-identical BMT performed at our institution (reticulocytes ≥1% on day 18 [mean; range 13 to 39]; bone marrow RBC precursors ≥15% on days 30 through 43; 1 of 53 patients unevaluable due to early rejection and death).

Clinical data and course of RBC recovery in PRA. All three patients with temporary PRA were of blood group O and received RBC-depleted BMT from HLA-matched sib-

Table 2. Number of Days to Achieve Peripheral Blood Cell Counts and No. of RBC Transfusions After Major ABO-Incompatible BMT

<table>
<thead>
<tr>
<th>PRA patients</th>
<th>Leukocytes*</th>
<th>Granulocytes*</th>
<th>Platelets*</th>
<th>Reticulocytes</th>
<th>RBC Transfusions (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt; 1.0</td>
<td>&gt; 0.5</td>
<td>&gt; 1.0</td>
<td>&gt; 50</td>
<td>≥ 1%</td>
</tr>
<tr>
<td>UPN 39</td>
<td>22</td>
<td>24</td>
<td>30</td>
<td>22</td>
<td>247</td>
</tr>
<tr>
<td>UPN 40</td>
<td>19</td>
<td>18</td>
<td>19</td>
<td>16</td>
<td>185</td>
</tr>
<tr>
<td>UPN 50</td>
<td>24</td>
<td>25</td>
<td>35</td>
<td>27</td>
<td>148</td>
</tr>
</tbody>
</table>

Patients with normal RBC recovery (n = 11) | 20.2 ± 5 | 23.4 ± 5 | 33.8 ± 12 | 25.4 ± 6 | 22.3 ± 3 | 5.4 ± 4 |

Upper part: Results in three cases of temporary pure PRA. Lower part: Mean values (± 1 SD) in patients with timely complete engraftment.

* × 10^9/L.
ling donors of group A. They were conditioned by fractionated TBI (6 x 2 Gy over 6 days), preceded by high-dose chemotherapy (one) or Cy (two). The total number of nucleated marrow cells transplanted was 4.0, 5.1, and 7.0 x 10^6 per kg body weight, respectively. Cyclosporine was used for GVH prophylaxis. The clinical course after BMT was uneventful except for localized herpes zoster infection on day 176 (UPN 39), transient interstitial pneumonia of undetermined cause on day 14 (UPN 40), and mild GVHD (grade 1, skin) on day 16 (UPN 50). There were no signs of viral infection and no exposure to drugs explaining PRA. The course of erythroid engraftment was as follows.

In UPN 39 (39-year-old woman, AML in first remission), no reticulocytes could be demonstrated up to day 247 after BMT. Analyses of bone marrow aspirates on days 3, 31, 98, and 192 showed the morphologic picture of PRA with less than 1% RBC precursors in an otherwise normal marrow. During that period, the patient required 29 units of RBC transfusions of recipient ABO-type. Ten of them were given as washed RBC in saline and the remaining as leukocyte-poor RBC concentrates. Reticulocytes started to rise from day 247 onward (Fig 1). Hemoglobin blood levels remained above 10 g/dL after day 260 and were normal from day 295 onward. Bone marrow analysis on day 295 was normal with 28% erythropoiesis. At the same time, the complete change to donor ABO-type (group A, no mixed field pattern of agglutination with anti-A test sera) was documented. The patient is still in remission with normal blood counts and bone marrow morphology more than 40 months after BMT.

In all three patients with temporary PRA, no clinical or laboratory signs of hemolysis could be demonstrated at the time of reticulocyte recovery or after completion of RBC engraftment. After normalization of hemoglobin blood levels, reticulocyte counts returned to normal. With the exception of UPN 39, none of our PRA patients received any other treatment, such as steroids, aimed at induction or acceleration of RBC engraftment.

Serologic findings. Before BMT, the direct antiglobulin test was negative in all three patients and their ABO-incompatible donors, and no irregular complete or incomplete RBC antibodies could be demonstrated. The pretransplant titer of anti-A agglutinin was very high in UPN 39 (1,024; IgG 1,024). The other two patients showed titers in the usual range (UPN 40: 32; IgG 128; UPN 50: 64; IgG 4).
After BMT a decrease of the agglutinin titers was observed over the first few weeks but this decline was not sustained thereafter (Figs 1 through 3). To the contrary, the titers increased again 5 to 10 weeks after BMT and persisted at relatively high levels for many weeks to decrease permanently only after 19 to 28 weeks. Anti-A agglutinins (as well as IgG) were no longer detectable in undiluted patient's sera after day 295 (UPN 39) and day 363 (UPN 40). In UPN 50, anti-A agglutinin persisted (titer ≤2) and anti-A IgG antibody could be demonstrated in eluates obtained from his RBC up to day 540 after BMT, but not after day 595. Repeat direct antiglobulin tests were negative throughout the observation period in UPN 39 and 50. In UPN 40, a transiently positive antiglobulin test was observed simultaneously with the increase of the reticulocyte count. Broad spectrum and monospecific anti-IgG and anti-C3 antiglobulin sera showed equally positive results. An eluate of the patient’s RBC was nonreactive in the indirect antiglobulin test, with 24 of 24 RBC of two RBC test panels of blood group O, but was strongly positive with 6 of 6 normal donor RBC of group A₁, and positive with 3 of 3 of group A₂.

To reduce the high anti-A titer, UPN 39 was subjected to six large-volume plasma exchanges within 4 weeks. Although a decrease by 2 to 3 doubling dilutions could be achieved immediately at the end of each plasma exchange, the agglutinin titer returned to ≥64 within a few days. A bone marrow analysis at the end of this series of exchanges showed 0.5% of erythropoiesis and no reticulocytes appeared in the peripheral blood.

Compared with PRA patients, the evolution of agglutinin titers and reticulocyte counts was as follows in patients with timely RBC engrafting. Agglutinins showed a progressive decrease and titers persistently ≤16 were observed from day 32 onward. At the same time period, reticulocyte recovery occurred (mean: day 22, range 15 to 36). In 6 of 8 patients surviving at least 2 months, agglutinins vanished completely within 50 days (range 27 to 49), whereas in 2 of 8 patients anti-A agglutinin persisted at low titers (≤4) up to 4 and 8 months after BMT, despite normal reticulocyte recovery.

Possible factors predicting PRA. To identify potential risk factors for delayed RBC engraftment associated with ABO-incompatible BMT, we analyzed the serologic findings
in 4 of 11 ABO incompatible BMT recipients with timely RBC recovery. The remaining seven patients prepared for BMT by plasma exchanges have not been considered because their agglutinin titers had been lowered artificially before BMT (baseline titers: 32 to 512; 5 of 7 ≥ 64; at BMT: 0 to 4, IgG 0 to 4). These four patients with timely RBC recovery received RBC-depleted grafts as PRA patients did. Their agglutinin and IgG titers before BMT were 64/64, 64/4, 16/8, and 4/8, respectively (as compared with 1.024/1.024, 32/128, and 64/4 in PRA patients). Hence, high pretransplant agglutinin and/or IgG titers did not clearly discriminate between patients developing PRA and those with timely RBC recovery (IgM: r = .73; IgG: r = .76). During the first weeks after BMT, the decrease of agglutinin titers was similar in patients with PRA (Figs 1 through 3) and patients with normal RBC recovery (mean decrease 2.3 [range 0 to 5] doubling dilutions at the end of the first week, 3.3 [1 to 5] after the second, and 3.9 [1 to 6] after the third week). It was only at this later period after BMT when a striking difference in the course of agglutinin titers was observed with the aforementioned rise in agglutinin titers in PRA patients. Accordingly, rising and persistently high agglutinin titers predict failure of timely RBC recovery.

The posttransplant course of agglutinins indicate the persistence of functional B-lymphocytes of recipient origin. Therefore, bone marrow cells and peripheral lymphocytes were analyzed for mixed chimerism. In UPN 40, complete chimerism was documented by the presence of donor sex chromosome (46 XY) in all marrow mitosis on day 31 (5 of 5 mitosis), day 126 (13 of 13) and day 247 (4 of 4). DNA fingerprints of bone marrow cells and lymphocytes also showed a complete change from recipient-type pretransplant to donor-type on days 31 and 184 after BMT (UPN 39), and on day 101, respectively (UPN 40). No chromosome or DNA studies were available in UPN 50.

PRA was observed in 3 of 9 patients receiving cyclosporine for GVH prophylaxis, but in 0 of 5 receiving methotrexate (P = .23). There was no correlation between cytoplasmic dosage or whole blood trough levels and reticulocyte recovery. In patients with PRA, reticulocyte eventually occurred 83 days (UPN 39) and 10 days (UPN 40) after conclusion of cyclosporine prophylaxis, while UPN 50 still received 2 mg/kg/d of cyclosporine at the time of recovery.

DISCUSSION

Our experience confirms previous observations, indicating that major ABO incompatibility does not significantly affect the ultimate outcome of BMT. In accordance with most studies, we found a timely recovery of granulocyte and platelet counts after ABO-incompatible BMT without increase of graft rejections. Although a short delay in reconstitution of reticulocyte counts does not seem uncommon, prolonged PRA comparable with that reported here has only rarely been described, and exclusively after major ABO-incompatible BMT. Because no other explanation for erythroid engraftment failure (such as irregular RBC alloantibodies, autoimmune hemolysis, chronic viral infection, GVHD, or drugs) was identified, this complication, which also in our series never occurred after ABO-identical BMT, appears to be associated with major ABO incompatibility.

Delay of RBC engraftment after ABO-incompatible BMT has been attributed to high pretransplant agglutinin titers by several investigators whereas no apparent correlation of the two findings has been reported by others. While the ABO agglutinin titers observed in these series were admittedly heterogeneous, our findings do not support a concept that pretransplant agglutinin titers alone predict the development of PRA.

However, we observed that a posttransplant rise and persistence of high anti-A agglutinin titers is associated with a delay of RBC engraftment. Persistence of high titers was strictly related with the absence of reticulocytes in the peripheral blood and erythroid precursors in the marrow. Similarly, impaired erythroid development until agglutinins fell to low titers has been observed by others. We therefore assume that the occasional delay in erythroid engraftment after ABO-incompatible BMT is causally linked in as yet unknown ways to agglutinins persisting at relatively high levels in the posttransplant period. It remains open whether these antibodies alone induce sufficient cytoxicity to the relevant erythroid precursors or merely reflect an additional immunopathology.

Reasons for a reappearance and long-lasting persistence of high agglutinin titers after high-dose chemoradiotherapy and allogeneic BMT in a few cases is unknown. A passive transfer of agglutinins by transfusions is excluded in our cases. Because our PRA patients were not subjected to pretransplant plasma exchanges to reduce their agglutinin titers, their total body antibody pool was presumably higher than in patients prepared by plasma exchanges, possibly a contribution to a longer antibody persistence. Similarly, most PRA cases published so far also received RBC-depleted marrow and were not prepared by plasma exchange. Moreover, Jin et al found a significantly prolonged requirement for RBC transfusions in 19 recipients of RBC-depleted marrow compared with 25 patients who underwent antibody depletion. However, considering the long period of high antibody levels in PRA patients, persistent antibody synthesis by immunocompetent cells of recipient type must be assumed in our PRA patients. Sniecinski et al identified mixed chimerism in 2 of 4 PRA cases by immunoglobulin allotypes or sex chromosome analyses. Persistence of host lymphocytes could not be documented by sex chromosome analyses and/or DNA typing in two of our patients. Clearly, this findings does not exclude the presence of recipient cells up to a range of 5% to 10% in the bone marrow or peripheral blood.

The potential role of cyclosporine in facilitating donor-derived antibody synthesis after minor ABO-incompatible BMT has been suggested. Similarly, its effect in maintaining recipient-derived agglutinins in major ABO-incompatible BMT has recently been shown. Cyclosporine is a powerful immunosuppressive agent acting primarily on T-lymphocyte proliferation in response to primary antigen stimulation. However, proliferation is not impaired when sensitized lymphocytes are re-exposed to the antigen in the
presence of cyclosporine, a situation applying to ABO-incompatible BMT. In the present context it is particularly intriguing that all cases of long-lasting PRA reported so far, including ours, were seen under cyclosporine treatment. However, we have no explanation as to why not all cyclosporine-treated patients developed PRA after ABO-incompatible BMT. Since both the small number of cases and insufficient knowledge about the mechanisms involved preclude a definite conclusion, it seems warranted to further study the potential role of cyclosporine in the pathogenesis of PRA after ABO-incompatible BMT.

The optimal treatment of PRA characterized by increasing posttransplant antibody titers is not known. In one patient, a rapid RBC recovery was obtained after early and effective antibody clearance by a series of daily large-volume plasma exchanges. Less-intensive plasma exchanges later on the post-BMT course were unsuccessful in another patient as well as one of our own. Corticosteroids administered empirically to three PRA patients were also without apparent benefit. Because RBC engraftment and reticulocyte recovery ultimately occurred spontaneously in most patients, management generally requires only the transfusion of RBCs. Without formal proof, we feel that in patients with PRA and persistence of high-agglutinin titers beyond 2 months after BMT, a rapid dose reduction or withdrawal of cyclosporine should be considered, and the risk of GVHD balanced against the risks of prolonged requirement of RBC substitution.

REFERENCES


